

REMARKS

This Amendment is responsive to the Office Action dated January 19, 2001. Claims 1-6 and 8-9 have been amended. New claims 10-14 have been added. Claims 1-14 are currently pending in the present application.

The applicant filed an Information Disclosure Statement and form PTO-1449 in Paper No. 6 which was acknowledged by the Examiner in the Office Action. However, the Examiner did not return the initialed form PTO-1449 form to the applicant. Accordingly, the applicant requests that the Examiner return the initialed form PTO-1449.

Claims 1-9 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite. Each of the bases for this rejection is addressed separately below.

Claim 1 was rejected on the basis that it failed to recite active method steps. Claim 1 has been rewritten to overcome this rejection.

Also, the parentheses have been removed from claim 1. Parentheses are a common symbol used by skilled chemists in naming certain chemical compositions. However, to eliminate any possible ambiguity in the claims, the parentheses have been eliminated from all of the claims. It is considered that this amendment does not change the meaning or scope of the claims.

Claim 1 has also been revised to eliminate use of the word "provided" to obviate the Examiner's objection.

In addition, claim 1 has been split into claim 1 and new claim 10 to overcome the rejection that the claim cannot both "comprise" or "be in contact with" a sensitizing moiety. Claim 1 now recites "comprises" and new claim 10 recites "is in contact with." A discussion of these features can be found on page 2, lines 27-30 of the specification. Claim 5 has been similarly amended.

According to the Examiner, claim 3 has been considered indefinite for using the parentheses. In claim 3, the parentheses are used to abbreviate two different materials as is the common practice in

chemistry. For example, the phrase (thio)-indigo derivates means both thioindigo derivatives and indigo derivatives. The claim has been amended accordingly to remove the parentheses and has been rewritten to include both possibilities in order to retain the same meaning and scope of the claim.

According to the Examiner, claim 3 is considered indefinite because of the term "derivatives." However, the term "derivatives" should not raise the issue of indefiniteness because it is well defined in the specification. In page 4, line 24, the specification says, "These compounds and derivatives are well-known to those skilled in the art". The specification further illustrates the meaning of "derivatives" by several examples of such derivatives. Therefore, the term "derivatives" is definite in the context of this application. The same arguments apply to claims 4 and 7. Withdrawal of this rejection for claims 3, 4, and 7 is respectfully requested.

According to the Examiner, claim 4 is considered vague and indefinite in reciting, "the ligand is a composition comprising a compound comprising." The composition and the compound are not the same. Thus the composition comprises a compound as one of its constituents. The compound, in turn, comprises specific complexing moieties. To further clarify this point, the claim has been slightly amended to retain the same meaning but to improve its readability.

According to the Examiner, claim 4 is considered to have an improper antecedent basis problem. This problem has been corrected by amending claim 4 to read "the sensitizing moiety."

According to the Examiner, claim 5 is considered to be indefinite in using parenthetical symbols. This problem has been corrected by a similar amendment as was made in claim 3.

According to the Examiner, claims 6 and 9 are considered to be indefinite in reciting the term "suitable." Claims 6 and 9 have been amended to replace "suitable for" with "capable of" in order to overcome this rejection. Capability of detecting luminescence in the wavelength range of 800-1600 nm can be objectively determined by a skilled person.

According to the Examiner, claim 9 is considered to have an improper antecedent basis problem. To correct this problem, claim 9 was amended to read as "the apparatus as claimed in claim 6."

Claims 1-9 have been rejected under 35 U.S.C. §102 (e) as being anticipated by Kardos et al (US 6,159,686). This rejection is respectfully traversed and reconsideration is requested for the reasons that follow.

Kardos et al discloses a two-photon process, which is unmistakably indicated at lines 32-34 of col. 30. Kardos et al further discloses that Rhodamine can be excited at 650-700 nm using two photons (col. 30, lines 47-48). This means that the two photons required to provide the energy required to excite the complex in Kardos et al are in the 325-350 nm wavelength range. Had Kardos et al used a one-photon process, the absorbance would be in the 325 -350 nm range to achieve the same energy level. In addition, the two-photon process is relatively inefficient since the chance that two photons are absorbed by the complex at the same time in order to excite the complex to the proper energy level is relatively small. A two-photon process can, therefore, only be used when high excitation intensities are used (see attached article "Laser Spectroscopy" by Wolfgang Demtröder, Springer-Verlag, 1982, page 438 which states that pulsed lasers are generally used to deliver sufficiently large peak powers for two-photo excitations). The advantage of the presently invented one-photon process is that an efficient process is obtained at normal excitation intensities. Therefore, Kardos does not disclose or suggest a one-photon process using light with a wavelength in the range of 400-1000 nm region. For this reason, Kardos et al does not anticipate the present invention as claimed in claims 1-9, as amended.

The same argument applies to new claims 10-14 as well. Therefore, the present invention as recited in amended claims 1-9 and newly added claims 10-14 is clearly novel over Kardos. Favorable consideration and withdrawal of the rejection is respectfully requested.

Claims 1-4 and 7 have been rejected under 35 U.S.C. §102 (e) as being inherently anticipated by Wieder et al (US 5,830,769). This rejection is respectfully traversed and reconsideration is requested for the reasons, which follow.

Wieder does not disclose specific ranges of light wavelengths for excitation. Furthermore, the Examiner has provided no evidence in support of the conclusion that the radiation used by Wieder is within the claimed region (400 nm –1000 nm). Therefore, Wieder does not anticipate the present invention as claimed in claims 1-9, as amended. Favorable consideration and withdrawal of the rejection is respectfully requested.

Claims 5-6 and 8-9 have been rejected under U.S.C. §103 (e) as being unpatentable over Wieder et al. (US 5,830,769) in view of Kardos et al (US 6, 159, 686).

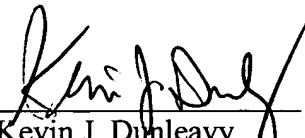
Neither Wieder et al nor Kardos et al discloses or suggests "a sensitizing moiety which absorbs in the 400-1000 nm region," which is recited in claims 5-6 and 8-9.

Therefore, the kit of the present invention as claimed in claims 5-6 and 8-9 is not obvious to one of ordinary skill in the art from a combination of Wieder with Kardos, since one element of the kit, the sensitizing moiety is not taught or suggested by either Wieder or Kardos. Favorable consideration and withdrawal of the rejection is respectfully requested.

In addition, a copy of the article "Laser Spectroscopy" by Wolfgang Demtröder, Springer-Verlag, 1982, pages 436-441 is enclosed herewith for your convenience.

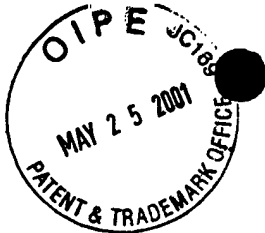
In view of the above amendments to the claims, as well as the foregoing remarks, Applicant respectfully submit that all of the pending claims are in condition for allowance and respectfully requests a favorable Office Action so indicating.

Respectfully submitted,


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1. (Amended twice) A method for detection of an analyte in a test sample [by a specific binding reaction among the analyte, a specific binding partner for the analyte, and an (immuno)reactant provided with a label, wherein that the label is lanthanide ion-ligand complex wherein the lanthanide ion is neodymium(III) ion (Nd^{3+}), ytterbium(III) ion (Yb^{3+}), or erbium(III) ion (Er^{3+}) and the ligand comprises or is in contact with a sensitizing moiety which absorbs in the 400-1000 nm region, and preferably in the 400-800 nm region.]comprising the steps of:

preparing a lanthanide ion-ligand complex by contacting a lanthanide ion ^{with} and a ligand, wherein the lanthanide ion is selected from the group consisting of neodymium (III) ion, ytterbium (III) ion (Yb^{3+}) and erbium (III) ion (Er^{3+}), and wherein the ligand comprises a sensitizing moiety, which absorbs light in the 400 –1000 nm region;

labeling an immunoreactant with the lanthanide ion-ligand complex by contacting the immunoreactant with the lanthanide ion-ligand complex to form a labeled immunoreactant;

mixing the analyte, a specific binding partner for the analyte and the labeled immunoreactant to form a mixture;

irradiating the mixture with light having a wavelength ranging from 400 nm to 1000 nm; and
measuring an emitted luminance from the mixture.

correlation?

2. (Amended) The method [according to claim 1 wherein the lanthanide ion is] as claimed in claim 1, wherein the lanthanide ion is selected from the group consisting of neodymium (III) ion (Nd^{3+}) [or]and ytterbium (III) ion (Yb^{3+}).

3. (Amended twice) The method [according to claim 1 or 2 wherein the]as claimed in any one of claims 1, 2, 10 and 11, wherein the sensitizing moiety is selected from the group consisting of fluorescein

derivatives; triphenylmethane derivatives; porphyrin derivatives; rhodamine derivatives; phenothiazine derivatives; phenoxazine derivatives; coumarin derivatives; acridin derivatives; [(thio)- indigo derviatives] thio-indigo derviatives; indigo derivatives; carbocyanine derivatives; squaraine derivatives; [and (na)phthalocyanine derivatives] naphthalocyanine derivatives; and phthalocyanine derivatives.

4. (Amended twice) The method [according to] as claimed in any one of claims 1 and 10, wherein the ligand is a composition [comprising a compound comprising] which comprises, as one of its constituents, a compound which comprises an element selected from the group consisting of oxygen, nitrogen, phosphorous, and sulfur moieties which have complexing ability towards Nd (III), Yb (III), or Er (III) ions, [in particular polyaminocarboxylic acid, pyridinedicarboxylic acid, or a derivative thereof] and the [a] sensitizing moiety is selected from selected from the group consisting of fluorescein derivatives; triphenylmethane derivatives; porphyrin derivatives; rhodamine derivatives; phenothiazine derivatives; phenoxazine derivatives; coumarin derivatives; acridin derivatives; [(thio)indigo derivatives] thio-indigo derivatives; indigo derivatives; carbocyanine derivatives; squaraine derivatives; [and (na)phthalocyanine derivatives] naphthalocyanine derivatives; and phthalocyanine derivatives.

5. (Amended twice) A kit for detection of an analyte in a test sample comprising:

a specific binding partner for the analyte;

an immunoreactant; and [provided with]

a label wherein the label is a lanthanide ion-ligand complex formed by contacting a lanthanide ion and a ligand, [and] wherein the lanthanide ion is selected from the group consisting of neodymium(III) ion (Nd^{3+}), ytterbium(III) ion (Yb^{3+}), [or] and erbium(III) ion (Er^{3+}), and wherein the ligand comprises [or is in contact with] a sensitizing moiety which absorbs light in the 400-1000 nm

region[, and wherein the specific binding partner and the labeled immunoreactant are optionally attached to a carrier].

6. (Amended twice) An apparatus for detection of an analyte in a test sample comprising:

the kit of any one of claims 5, 12, 13 and 14;

a light source in the 400-1000 nm range ; and

a detector, which is capable of [suitable for]detecting luminescence in the 800-1600 nm.

8. (Amended) The kit of any one of claims 5, 12, 13 and 14, wherein the sensitizing moiety absorbs in the 400-800nm region.

9. (Amended) The [An] apparatus as claimed in claim 6, wherein the detector is capable of [suitable for] detecting luminescence in the 800-1100nm range.

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Wolfgang Demtröder

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P. 2

Fig. 8.19. Generation of double-resonance signals by transfer of population modulation via fluorescence or collisions

assignment of conventional absorption spectra. The $\chi_{\text{L}}^{\text{I}} \rightarrow \chi_{\text{L}}^{\text{II}}$ transitions of Mg_2 are an example which has been studied this way. Figure 8.3B shows the GDR spectrum [8.90a]. Note the significant simplification of the complex linear absorption spectrum (see also [8.50b]).

Since the intensity $I_1(\lambda)$ of the fluorescence emitted from level 2 has the same modulation phase as ΔH_2 , the population modulation ΔH_2 is partly transferred to all terminating levels k (Fig. 8.39). The modulation ΔH_k of level k is

1

At higher pressures in the sample cell the population modulations Δn_1 or Δn_2 may be transferred by collisions to neighboring levels and additional lines appear in the double-resonance spectrum which are excited by L_2 from this collisional modulated levels i (see Sect. 12.2). Such effects increase the number of lines in the double-resonance spectrum and may reduce the simplicity of the method. Measuring the pressure dependence of the QM1 signals allows one, however, to distinguish between direct and collisional-induced lines.

8.10 Raman Scattering Spectroscopy

The stepwise excitation, discussed in Sect. 8.B, can be interpreted as two successive one-photon excitations. In this section we consider the *one-dimensional* absorption of two or more photons by a molecule which undergoes a transition $E_1 \rightarrow E_2$ with $(E_2 - E_1) = h\nu_1$. The photons may either come from a single λ -

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P. 2

Case described in period.
 combination of (c) and (d) →
 excitation through virtual
 level, followed by fluorescence
 very low probability
 (8.43)

$$A_{if}^{(2)} = \frac{\gamma_{if}}{[\omega_f - \omega_1 - \omega_2 - \frac{1}{2}(\mathbf{k}_1 + \mathbf{k}_2)]^2 + (\gamma_{if}/2)^2} \sum_k \frac{R_{ki} \cdot \mathbf{e}_1 \cdot R_{kf} \cdot \mathbf{e}_2}{[\omega_k - \omega_1 - \mathbf{k}_1 \cdot \mathbf{v}] [\omega_k - \omega_2 - \mathbf{k}_2 \cdot \mathbf{v}]} + \frac{R_{ki} \cdot \mathbf{e}_2 \cdot R_{kf} \cdot \mathbf{e}_1}{[\omega_k - \omega_2 - \mathbf{k}_2 \cdot \mathbf{v}] [\omega_k - \omega_1 - \mathbf{k}_1 \cdot \mathbf{v}]} \cdot \frac{1}{\gamma_{if}^2}$$

The first factor gives the spectral line profile of the two-photon transition. It corresponds exactly to that of a single-photon transition of a moving molecule at a center frequency $\omega_{if} = \omega_1 + \omega_2 + \frac{1}{2}(\mathbf{k}_1 + \mathbf{k}_2)$ with a homogeneous linewidth γ_{if} (see Sects. 3.1, 6). Integration over all molecular velocities \mathbf{v} gives a Voigt profile with a linewidth which depends on the relative orientation of \mathbf{k}_1 and \mathbf{k}_2 . If both light waves are parallel, the Doppler width which is proportional to $|\mathbf{k}_1 + \mathbf{k}_2|$ becomes maximum and is in general large compared to the homogeneous width γ_{if} . For $\mathbf{k}_1 = -\mathbf{k}_2$ the Doppler broadening vanishes and we obtain a pure Lorentzian line profile with a homogeneous linewidth γ_{if} provided that the laser linewidth is small compared to γ_{if} . This "Doppler-free two-photon spectroscopy" will be discussed in Sect. 10.6.

Because the transition probability (8.43) is proportional to the product of the intensities $I_1 I_2$ (which has to be replaced by I^2 in case of a single laser beam), pulsed lasers are generally used which deliver sufficiently large peak powers. The spectral linewidth of these lasers is often comparable to or even larger than the Doppler width and the denominators $(\omega_k - \omega_i - \mathbf{k} \cdot \mathbf{v})$ can then be approximated by $(\omega_k - \omega_i)$.

The second factor describes the transition probability for the two-photon transition. It can be derived quantum mechanically by second-order perturbation theory (see for example [8.94]). This factor contains a sum of products of matrix elements $R_{ki} R_{kf}$ for transitions between the initial state i and intermediate molecular levels k or between these levels k and the final state f (see [2.98]). The summation extends over all molecular levels k . The denominator shows, however, that only those levels k which are not too far off resonance with one of the Doppler-shifted laser frequencies $\omega_k = \omega_i - \mathbf{k} \cdot \mathbf{v}$ ($\omega_k - \omega_i \approx \omega_f - \omega_i$) will mainly contribute.

Often a fictitious "virtual level" is introduced to describe the two-photon transition by a symbolic two-step transition $E_i \rightarrow E_f$ (Fig. 8.40). Since the two possibilities

- a) $E_i + \hbar\omega_1 \rightarrow E_f$, $E_f + \hbar\omega_2 \rightarrow E_f$ (first term)
- b) $E_i + \hbar\omega_2 \rightarrow E_f$, $E_f + \hbar\omega_1 \rightarrow E_f$ (second term)

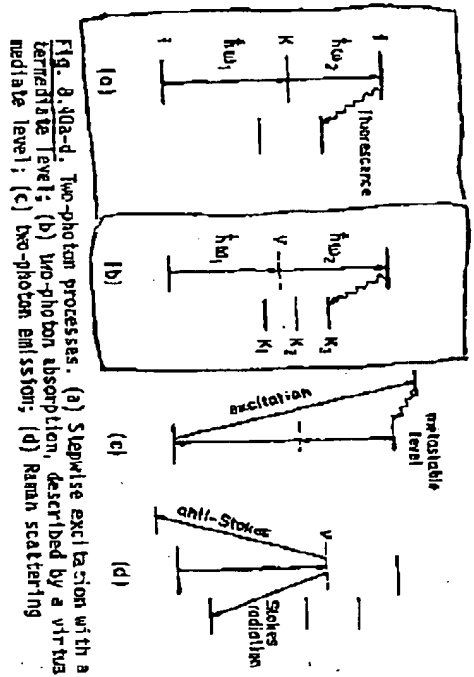


Fig. 8.40a-d. Two-photon processes. (a) Stepwise excitation with a real intermediate level; (b) two-photon absorption, described by a virtual intermediate level; (c) two-photon emission; (d) Raman scattering

lead to the same observable result, namely the excitation of the real level E_f , the total transition probability for $E_i \rightarrow E_f$ is the square of the sum over both probability amplitudes, as indicated by the sum over the two terms in (8.43).

Often the frequencies ω_1 and ω_2 can be selected in such a way that the virtual level is close to a real molecular eigenstate. This greatly enhances the transition probability and it is therefore generally advantageous to excite the final level E_f by two different photons with $\omega_1 + \omega_2 = (E_f - E_i)/\hbar$, rather than by two photons out of the same laser with $2\omega = (E_f - E_i)/\hbar$.

The second factor in (8.43) describes quite generally the transition probability for all possible two-photon transitions such as the Raman scattering or the two-photon absorption and emission. Figure 8.40 illustrates schematically these three two-photon processes. The important point is that the same selection rules are valid for all two-photon processes. Equation (8.43) shows that both matrix elements R_{ki} and R_{kf} must be nonzero to give a nonvanishing transition probability $A_{if}^{(2)}$. This means that two-photon transitions can only be observed between two states i and f which are both connected to intermediate levels k by allowed single-photon optical transitions. Because the selection rule for single-photon transitions demands that the levels i and k or k and f have opposite parity, the two levels i and f , connected by a two-photon transition, must have the same parity. In atomic two-photon spectroscopy $s \rightarrow s$ or $s \rightarrow d$ transitions are allowed, and also $\bar{s}g \rightarrow \bar{s}g$ transitions, for example, in diatomic homonuclear molecules.

It is therefore possible to reach molecular states which cannot be populated by single-photon transitions from the ground state. In this regard two-

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utilize the group theoretical techniques originally developed for Raman scattering to analyze the symmetry properties of the excited states.

8.10.2 Applications of Multiphoton Absorption to Atomic and Molecular Spectroscopy

The first two-photon absorption in the molecular gas phase was observed in benzene by KUCHTERSSER et al. (8.96). An extensive two-photon excitation spectrum of the $1_{1g} \rightarrow 1_{2g}$ transition in benzene (including the hot bands (8.97) allowed the first assignment of new molecular states. The two-photon absorption is monitored by the fluorescence emitted from the upper excited level. Two-photon spectroscopy in the collisionless low-pressure gas phase together with polarization studies enables one to study in detail the spectroscopy and the dynamics of two-photon prepared states not accessible by one-photon absorption (8.98). However, polarization criteria as a method of assigning two-photon molecular spectra are more ambiguous than in atomic spectra and must take explicit cognizance of the rotational variations in the polarization ratios, because no unique polarization dependence can be expected for the different rotational lines of a vibrational band (8.99).

These studies of two-photon excitation spectra of benzene, naphthalene, and other organic molecules have been performed with nitrogen laser-pumped dye lasers oscillating around $\lambda = 500$ nm which yields two-photon excitations at about 40 000 cm^{-1} . Still higher states can be reached by using frequency-doubled pulsed dye lasers. With a primary photon energy of 2.2 eV ($\lambda = 564$ nm) the excitation energy of 8.8 eV is sufficiently high to excite states which emit vacuum ultraviolet fluorescence. While the exciting photons (4.4 eV) can still pass through quartz windows, the emitted fluorescence has to be monitored through windows which transmit in the uv range, such as MgF₂ windows. With this technique the highly excited states A_1' in CO and 1_{1g} in H_2 could be reached and high-resolution spectra for the S branch heads of the (9,0) band of CO and the (5,0) band of H_2 have been recorded (8.100). The rotational selection rules, which are $\Delta J = 0, \pm 1$ for single-photon transitions, are $\Delta J = 0, \pm 1, \pm 2$ for two-photon transitions, where the value of ΔJ depends on the polarization of the incident radiation (see Fig. 8.41). While for two-photon transitions the parity of the excited state is the same as that of the absorbing ground state, three-photon transitions excite states with the same parity as single-photon transitions.

Both methods therefore complement each other in high-resolution laser spectroscopy in the vacuum ultraviolet region, where single-photon spectroscopy is impeded by lack of tunable lasers. With intense light of a pulsed

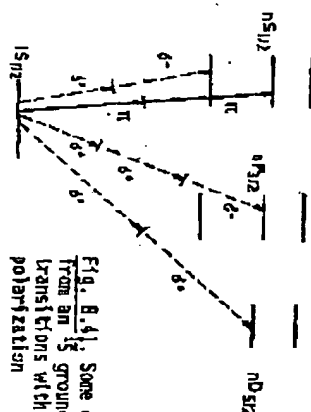


Fig. 8.41: Some excited atomic states, accessible from an $1s$ ground state by two- or three-photon transitions with linear (—) and circular (---) polarization

photon absorption spectroscopy is complementary to linear absorption spectroscopy, and its results are of particular interest, because they yield information about states, which often had not been found before. It is not seldom that excited molecular states are perturbed by nearby states of opposite parity and it is generally difficult to deduce the structure of these perturbing states from the degree of perturbations, while two-photon spectroscopy allows direct access to such states. Since the matrix element $R_{jk} = \langle j | \hat{A} | k \rangle$ and $R_{jk} = \langle j | \hat{A} | k \rangle$ depend on the polarization characteristics of the incident radiation, it is possible to select the accessible upper states by a proper choice of the polarization. While for single-photon transitions the local transition probability (summed over all M sublevels) is independent of the polarization of the incident radiation (see (2.35b)), there is a distinct polarization effect in multiphoton transitions, which can be understood by applying successively known selection rules to the two matrix elements in (8.43). For example, two parallel laser beams, which both have right-hand circular polarization, induce two-photon transitions in atoms with $\Delta l = 2$. This allows for instance $s \rightarrow d$ transitions but not $s \rightarrow p$ transitions. When a circularly polarized wave is reflected back on itself, the right-hand circular polarization changes into a left-hand one and if a two-photon transition is induced by one photon from each wave, only $\Delta l = 0$ transitions are selected. Figure 8.41 illustrates the different atomic transitions which are possible by multiphoton transitions with linearly polarized light and with right or left circularly polarized light.

Different upper states can be therefore selected by a proper choice of the polarization. In many cases it is possible to gain information about the symmetry properties of the upper states from the known symmetry of the ground state and the polarization of the two light waves (8.93). Since the selection rules of two-photon absorption and Raman transitions are identical, one can